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The *Toxoplasma gondii*-Shuttling Function of Dendritic Cells Is Linked to the Parasite Genotype▽

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Following intestinal invasion, the processes leading to systemic dissemination of the obligate intracellular protozoan *Toxoplasma gondii* remain poorly understood. Recently, tachyzoites representative of type I, II and III *T. gondii* populations were shown to differ with respect to their ability to transmigrate across cellular barriers. In this process of active parasite motility, type I strains exhibit a migratory capacity superior to those of the type II and type III strains. Data also suggest that tachyzoites rely on migrating dendritic cells (DC) as shuttling leukocytes to disseminate in tissue, e.g., the brain, where cysts develop. In this study, *T. gondii* tachyzoites sampled from the three populations were allowed to infect primary human blood DC, murine intestinal DC, or in vitro-derived DC and were compared for different phenotypic traits. All three archetypical lineages of *T. gondii* induced a hypermigratory phenotype in DC shortly after infection in vitro. Type II (and III) strains induced higher migratory frequency and intensity in DC than type I strains did. Additionally, adoptive transfer of infected DC favored the dissemination of type II and type III parasites over that of type I parasites in syngeneic mice. Type II parasites exhibited stronger intracellular association with both $CD11c^+$ DC and other leukocytes in vivo than did type I parasites. Altogether, these findings suggest that infected DC contribute to parasite propagation in a strain type-specific manner and that the parasite genotype (type II) most frequently associated with toxoplasmosis in humans efficiently exploits DC migration for parasite dissemination.

The obligate intracellular parasite *Toxoplasma gondii* infects virtually any warm-blooded vertebrate and ~25% of the world's human population (27). Most infections generate few or no symptoms. Yet, acute infections are a concern in human medicine, since this opportunistic pathogen causes severe neurological complications in immunocompromised individuals, disseminated congenital infections in the developing fetus, and ocular manifestations in otherwise healthy individuals (27). After ingestion of the parasite, acute infection is characterized by the proliferation of fast-growing stages (tachyzoites) that rapidly disseminate and differentiate into slow-growing stages (bradyzoites) in peripheral tissues, where they may persist for the lifetime of the host (27). In contrast to pathogens that rely on uptake by host cells, *T. gondii* actively invades host cells, including cells of the immune system, and replicates in a nonfusigenic parasitophorous vacuole (45).

Mounting evidence indicates that dendritic cells (DC) play critical roles during *T. gondii* infection as early sources of protective interleukin-12 responses and mediators of antigen presentation (32, 35, 37, 41). In addition, based on their migratory properties (40) and permissiveness to *Toxoplasma* infection (6), DC have recently been identified as systemic carriers (Trojan horses) of *T. gondii* tachyzoites (2, 7, 30). Yet, the precise roles of DC in the pathogenesis of toxoplasmosis and other parasitic infections remain elusive (43).

While the global population structure of *T. gondii* awaits further elucidation (9), three different clonal lineages (I, II, and III) of *T. gondii* appear to predominate in Europe, North America, and Africa (31, 46, 50), with type II infections prevailing in humans (1, 16, 22–24). Despite limited genetic diversity, virulence in the mouse model is strictly associated with the parasite genotype. Infections with type I strains are lethal in mice (100% lethal dose = 1), whereas type II and III strains can result in controlled infections that persist in the host (46).

Propelled by their own active motility (12), type I strains have the advantage that they efficiently cross biological barriers during the initial phase of infection (3) and tend to exhibit faster replication and higher parasite loads than those of type II and type III strains (10, 36, 49). Yet, spatiotemporal analysis of dissemination in vivo indicates efficient dissemination of type II parasites, but less dramatic expansion of parasite loads at peripheral sites, compared to those of type I parasites (21). We recently described that *T. gondii* induces a migratory phenotype in DC that potentiates parasite dissemination (30). To assess the impact of DC on the dissemination of *T. gondii*, we compared the abilities of type I, II, and III strains to induce migration of DC in vitro and in vivo in an intraperitoneal (i.p.) infection model. The studies presented here suggest that transportation of parasites by infected DC has an impact on the establishment of infection in a strain-dependent fashion.

MATERIALS AND METHODS

Parasites and mice. *T. gondii* tachyzoites were maintained by serial 2-day passage in human foreskin fibroblast monolayers cultured in Dulbecco modified Eagle medium (Invitrogen) with 10% fetal calf serum, 20 μg/ml gentamicin, 2 mM L-glutamine, and 0.01 M HEPES, referred to as complete medium (CM). Parasite strains used include green fluorescent protein (GFP)-expressing *Toxoplasma* lines RH-LDM (3) (cloned from RH-GFPS65T [28]) and ME49-PTG (PTG-GFPS65T [28]) and red fluorescent protein (RFP)-expressing line PRU (37). The various strains and clinical isolates of genotypes I, II, and III have been

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previously described (3, 24, 46). Six- to eight-week-old C57BL/6 female mice (Taconic) were used. The Animal Studies Committee at the Karolinska Institutet approved all protocols involving animals.

Human DC generation and culture. Buffy coats from healthy blood donors were treated with a monocyte enrichment cocktail (RosetteSep; StemCell Technologies), followed by centrifugation on Lymphoprep (Axis-Shield PoC AS). The cell population obtained was composed mainly of $CD14^+$ (DakoCytomation) with <1% $CD3^+$/$CD19^+$ cells (BD), as evaluated by flow cytometry (FACSCalibur; BD). Monocyte-derived DC were generated as previously described (30). Primary human myeloid DC were isolated as previously described (33), with some modifications. Myeloid DC were enriched from RosetteSep-treated buffy coats using CD1c magnetic bead isolation (Miltenyi Biotec), followed by sequential separation on autoMACS cell sorter (Miltenyi Biotec). Isolated DC were $CD11c^+$, $CD14^-$, $CD80^{low}$, $CD86^{low}$, and HLA-DR^+ (BD). DC were cultured in CM supplemented with granulocyte-macrophage colony-stimulating factor (60 ng/ml; PeproTech) and used directly after isolation. The Regional Ethics Committee, Stockholm, Sweden, approved all protocols involving human cells.

Murine DC generation and culture. Murine bone marrow-derived DC were generated as previously described (30). Isolation of small intestine (SI) DC and Peyer's patches (PP) DC was performed as described previously (25, 26), with some modifications. Briefly, intestinal segments and PP were digested separately with 400 Mandl units/ml collagenase D (Roche) and 10 μg/ml DNase I (Roche) in RPMI 1640 containing 20 mM HEPES and 20 ng/ml granulocyte-macrophage colony-stimulating factor (PeproTech). After 45 to 90 min, EDTA was added, and supernatants were collected by filtration through a nylon mesh (40-μm pores; BD). Leukocytes were further enriched on an LSM 1077 lymphocyte separation gradient (PAA). Cells were isolated using CD11c magnetic beads and separated on LS MACS columns (Miltenyi Biotec). Purified cells were stained for expression of CD11c and major histocompatibility complex class II (MHC-II) (I-A/I-E; BD). To ensure high viability, cells were infected and evaluated for transmigration (4 h) directly after isolation.

Immunofluorescence staining. DC were fixed with 0.3% glutaraldehyde on poly-L-lysine-coated glass coverslips and permeabilized using 0.1% phosphate-buffered saline–Triton X-100 (Sigma). Cells were stained with Alexa Fluor-conjugated phalloidin (Invitrogen), mounted using Vectashield with DAPI (4′,6-diamidino-2-phenylindole) (Vector Laboratories), and assessed by epifluorescence microscopy (Leica DMRB).

Video microscopy. Cell motility analyses were performed with a spinning-disk confocal setup (Ultraview LCI-3 tandem scanning unit; Perkin Elmer, United Kingdom) on an Axiovert 200 M microscope (Carl Zeiss, Germany) connected to a charge-coupled-device camera (OrcaER; Hamamatsu, Japan). Cells were placed in a minichamber system (POCmini; LaCon, Germany) with a heating stage. Image acquisition and analysis of motility were performed with Openlab software (version 5.0.2) and Volocity software (Improvision Inc.).

Transmigration assays. Infection of cells, generation of parasite lysate, and quantification of migrated cells were conducted as previously described (30). Briefly, DC were plated at a density of 1×10^6 to 2×10^6 cells/well (12-well plate) and incubated for 2 to 6 h with freshly egressed tachyzoites at the indicated multiplicity of infection (MOI). Infection frequencies were evaluated using flow cytometry (FACSCalibur). Cells were transferred into Transwell inserts (pore size, 3 μm; BD) and incubated for 3 to 18 h at 37°C. Migrated cells were quantified in a hematocytometer and/or by flow cytometry as previously described (30).

Adoptive transfers and inoculations. Seven-day-old bone marrow-derived DC were incubated with freshly egressed tachyzoites for 6 h at an MOI of 1. Cell suspensions were normalized for parasite viability, host cell viability, and infection frequency as follows. Cells were sequentially washed (80 g for 10 min) to remove extracellular parasites (<4%) and to enrich them for intracellular parasites (>95%). The viability of intracellular parasites was evaluated by a plaquing assay (CFU per added parasite) as described previously (30). Propidium iodide staining (>90% propidium iodide-negative [PI^{neg}] cells) was used to determine host cell viability by flow cytometry. The infection frequency (40 to 60%; expressing GFP [GFP^+] or expressing RFP [RFP^+]) of DC was evaluated by flow cytometry or plaquing assay as indicated. The average number of parasites/infected cell (~1.2) was determined by epifluorescence microscopy. The inoculum size was normalized, taking into account the infection frequency and the number of parasites/infected cell (30). The total number of CFU injected in animals was confirmed by plaquing assay (CFU per added cell). Analysis of parasite load in extracted organs was assessed by plaquing assay. For coinoculations, DC were labeled with 8-bromomethyl-4,4-difluoro-3,5-bis-(2-thienyl)-4-boro-3a,4a-diaza-*s*-indacene (BODIPY; Invitrogen), according to the manufacturer's instructions. Infected DC or free parasites were normalized as described above and mixed shortly before i.p. inoculation in mice.

Isolation of $CD11c^+$ and $CD11c^-$ cells. Mesenteric lymph nodes (MLN) and spleens were extracted and treated with 400 Mandl units/ml collagenase D (Roche) and 10 μg/ml DNase I (Roche) in RPMI 1640 for 20 min. After filtration through a nylon mesh (40-μm pores; BD), cells were isolated using CD11c magnetic beads and separated on LS MACS columns. $CD11c^+$ cells (>90%) were further depleted of CD3, CD19, Gr1, and NK1.1 (BD) using Biotin Binder Dynabeads (CELLection; Dynal Biotech ASA). Isolated cell populations ($CD11c^+$ $CD3^-$, $CD19^-$, $Gr1^-$, and $NK1.1^-$ cells and $CD11c^-$ cells) and cell suspensions from the spleen and MLN were quantified by using a hematocytometer. The number of viable parasites (CFU) in each population was evaluated by plaquing assay. Frequency of infection was defined as the number of parasite CFU per cell added to the plaquing assay.

Statistical analyses. Statistical analyses were performed using GraphPad Prism (version 4.00; GraphPad Software, Inc.).

RESULTS

Frequency of *Toxoplasma*-induced transmigration of DC depends on parasite genotype. *Toxoplasma* can subvert the regulation of host cell motility and induce a dramatic increase in DC migration in vitro that may promote parasite dissemination in vivo (30). To determine the capacity of different parasite genotypes to induce DC migration, human monocyte-derived DC were infected with 19 different clonal lineages and clinical isolates and assessed in a Transwell system. All strains tested induced significant DC transmigration compared to that of uninfected DC (CM versus individual strains; $P < 0.05$; Student's *t* test) (Fig. 1A). Strains with a type II genotypic background induced significantly higher frequencies of DC transmigration than did strains of types I and III (Fig. 1A). Also, type III strains exhibited a tendency for stronger induction of DC transmigration than that exhibited by type I strains ($P = 0.067$; one-way analysis of variance [ANOVA]). Genotype-associated differences were not explained by variations in infection rates of DC for different strains or by lysis of host cells (data not shown). Similar transmigration patterns were observed with murine bone marrow-derived DC (Fig. 1B and data not shown). In sharp contrast to DC exposed to parasite lysate or lipopolysaccharide (30), DC infected by *Toxoplasma* rapidly (<3 h) responded with a strong migratory phenotype that was most prominent for DC infected by type II strains (Fig. 1B). Next, cell motility was analyzed by time-lapse microscopy. Infected DC exhibited a dramatically enhanced motility compared to that of uninfected DC, and type II parasites induced stronger hypermotility in DC than that induced by type I parasites (Table 1). Altogether, these data show that while all strains tested induce migration of both human and mouse in vitro-derived DC, type II strains exhibit a higher induction of DC migration in vitro.

Induction of transmigration in primary murine intestinal DC and human blood DC is strain specific. After oral ingestion, tissue cysts or oocysts rapidly convert to tachyzoites in the intestinal lamina propria (13, 14). Consequently, tachyzoite-infected $CD11c^+$ DC are found in the lamina propria and in circulation after oral infection (7). To assess the differences between genotypes for migratory induction of primary cells, DC from the SI lamina propria and subepithelial tissue and from PP were extracted and characterized. Purified DC expressed high levels of CD11c and MHC-II (Fig. 2A) and various levels of CD86 and CD11b (data not shown). Purified $CD11c^+$ MHC-II^+ DC were readily infected by type I and type II parasites (Fig. 2B), and transmigration of infected cells was

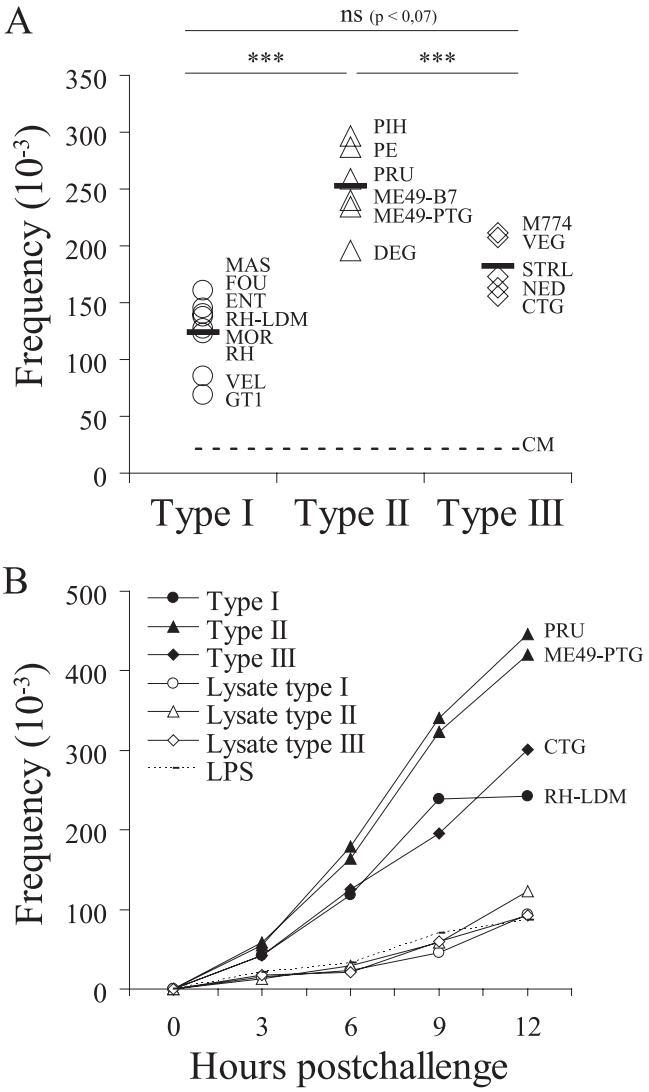

FIG. 1. Transmigration of DC infected with type I, II, and III parasite strains in vitro. DC were preincubated with freshly egressed tachyzoites for 6 h, and transmigration was measured in a Transwell system as described in Materials and Methods. (A) Transmigration of human monocyte-derived DC. DC were infected (MOI of 5) with tachyzoites from 19 strains and clinical isolates, indicated by abbreviations, followed by incubation for 18 h in Transwell inserts. Infection rates of DC were 60 to 85%. Circles, triangles, and diamonds represent the mean values from two to four experiments performed in triplicate for each strain. Solid bars represent the mean values for each group. Asterisks indicate significant difference ($P < 0.001$; one-way ANOVA). ns, not significant. (B) Kinetics of transmigration for murine bone marrow-derived DC after incubation with various *T. gondii* strains (MOI of 5), tachyzoite lysate (10 μg/ml), or lipopolysaccharide (100 ng/ml) in Transwell inserts. Abbreviations indicate parasite strains used. A representative experiment from two is shown.

studied in vitro. In accordance with observations on human monocyte-derived DC and murine bone marrow-derived DC (Fig. 1), type II parasites induced transmigration of SI DC and PP DC at higher frequencies than type I parasites (Fig. 2C and D).

To test the responses of primary human cells, myeloid DC from 10 healthy blood donors were purified. Between 85 and 98% of the purified $CD1c^+$ cells expressed high levels of CD11c, and the majority of the contaminating cells were $CD14^+$ or $CD19^+$ (Fig. 3A and data not shown). Both type I and type II parasites readily infected and replicated inside these cells (Fig. 3B). The infection rates were approximately 60 to 80%, depending on the donor, and were similar for type I and type II strains (data not shown). Although induction of DC transmigration varied substantially between donors, type II parasites induced a higher frequency of transmigration than type I parasites in 8 of 10 donors (Fig. 3C). Thus, infection with *Toxoplasma* can induce a migratory phenotype in primary human and murine DC, and this induction is higher for type II than for type I strains.

Dissemination of type I, II, and III parasites after adoptive transfer of *Toxoplasma*-infected DC. To further investigate the impact of infected and highly migratory DC on the dissemination of different parasite strains in vivo, mice were inoculated i.p. with 10^6 tachyzoites or tachyzoite-infected DC, and dissemination to the spleen and MLN was assessed after 16 h. In line with previous reports (3, 36), mice inoculated with free type I tachyzoites generated approximately 5 to 10 times higher parasite loads in the spleen and MLN than did mice inoculated with type II or type III tachyzoites (Fig. 4A and B). In contrast, when tachyzoite-infected DC were adoptively transferred to mice, similar numbers of type I, II, and III parasites reached the spleen and MLN (Fig. 4C and D). Compared to inoculations with free tachyzoites, adoptive transfer of tachyzoite-infected DC led to increased parasite loads for all three types. The most dramatic relative increase in parasite load was observed in mice infected with type II (~25-fold) and III (~10-fold) parasites, in contrast to a more modest increase in type I-infected mice (2- to 3-fold) (Fig. 4E and F). We conclude that, in this experimental setting, adoptive transfer of infected DC favored dissemination of type II and III parasites more than type I parasites.

Differential infection frequencies of DC during type I and type II infections. The observed differences in dissemination

TABLE 1. Migratory characteristics of DC infected with *T. gondii* type I or type II

Cell type	Distance migrated (μm)		Mean speed ($\mu m\ s^{-1}$)[c]
	Mean[a]	Maximum[b]	
Infected DC (type I)	152.9 (±53.4)	285.6	0.042 (±0.014)
Uninfected DC (type I)	33.1 (±15.0)	67.3	0.009 (±0.004)
Infected DC (type II)	196.9 (±68.6)	361.9	0.054 (±0.019)
Uninfected DC (type II)	37.3 (±17.0)	82.6	0.010 (±0.005)

[a] Mean distance migrated by DC ($n = 60$ [for each group]) recorded over 60 min using real-time confocal microscopy. Human monocyte-derived DC were preincubated with freshly egressed tachyzoites of type I (RH-LDM) or type II (PTG-GFP) at an MOI of 1 for 4 to 6 h immediately before visualization, as indicated in Materials and Methods. Infected DC exhibited significantly enhanced motility compared to uninfected DC in the same cell suspension ($P < 0.001$, Student's t test). DC infected with type II parasites exhibited significantly higher motility than did DC infected with type I ($P < 0.05$). Values are means (±standard errors of the means) of results from two independent experiments.

[b] Maximum distance recorded for individual cells for each group.

[c] Mean speed of DC ($n = 60$ [for each group]) during a 60-min recording. Infected DC exhibited significantly enhanced migratory speed compared to uninfected DC ($P < 0.001$). DC infected with type II parasites exhibited significantly higher speed than did DC infected with type I ($P < 0.05$). Values are means (±standard errors of the means) of results from two independent experiments.

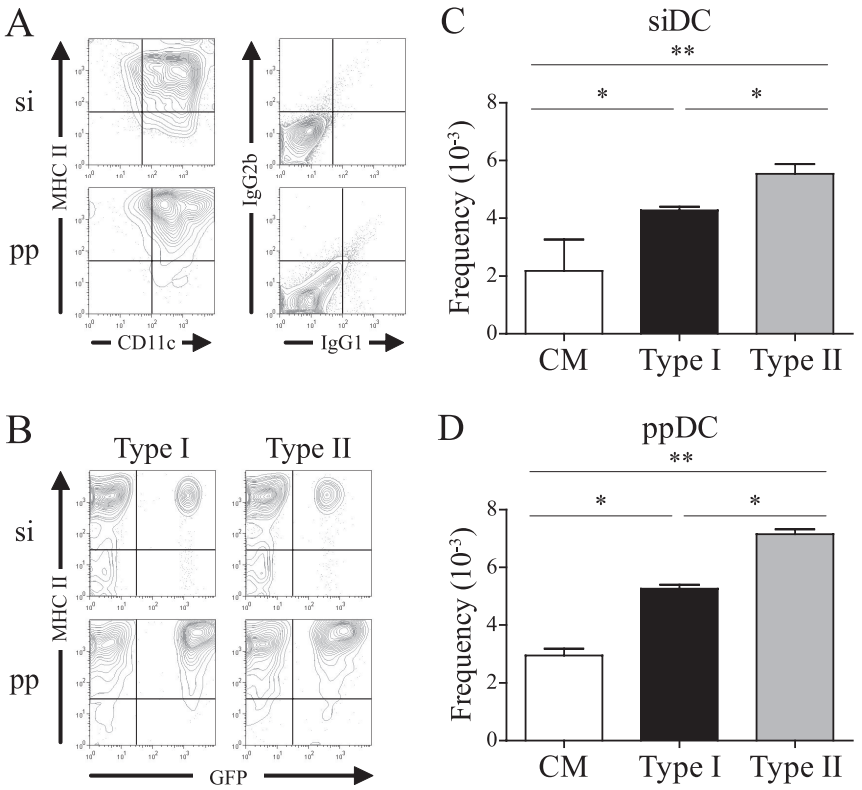

FIG. 2. Transmigration of primary SI DC and PP DC after *T. gondii* infection in vitro. Intestinal DC were isolated from C57BL/6 mice as indicated in Materials and Methods. (A) Flow cytometric contour plots show expression of CD11c and MHC-II (I-A/I-E) or isotype controls, after purification. (B) DC from the SI and PP, positively selected for CD11c, were infected (MOI of 1 to 3) with freshly egressed GFP-transfected tachyzoites (type I, RH-LDM; type II, ME49-PTG) in culture medium (CM) for 4 h and subsequently stained for MHC-II expression. Contour plots show viable (PI^{neg}) MHC-II and GFP double-positive cells, i.e., infected DC. (C and D) Bar diagrams show the transmigration frequency (mean ± standard deviation) of $CD11c^+$ SI DC and PP DC, respectively, after 4 h of incubation (MOI of 1) with type I (RH-LDM) or type II (ME49-PTG) parasites in Transwell inserts. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$; Student's *t* test). Data from a representative experiment performed in triplicate are shown.

efficiencies between *T. gondii* genotypes after adoptive transfers of infected DC motivated an analysis of the infection frequencies of DC and non-DC populations in vivo. C57BL/6 mice were challenged i.p. with 2×10^6 CFU of GFP-transfected type I (RH-LDM) or type II (ME49-PTG) tachyzoites. After 32 h, DC from spleens and MLN were purified based on CD11c expression and lack of CD3, CD19, NK1.1, and Gr1 expression and stained for MHC-II (Fig. 5A and data not shown). The infection frequencies of DC-rich $CD11c^+$ populations and $CD11c^-$ populations were subsequently evaluated by flow cytometry (Fig. 5A) and by plaquing assay (Fig. 5B). $CD11c^+$ cells exhibited a higher infection frequency than that exhibited by $CD11c^-$ cells, suggesting preferential infection of DC for both type I and type II genotypes (Fig. 5B). In line with the overall higher parasitic loads (Fig. 4A and B), mice infected with type I parasites exhibited significantly higher numbers of infected $CD11c^+$ and $CD11c^-$ cells than did mice infected with type II parasites ($P < 0.05$; Student's *t* test) (Fig. 5B). Importantly, when the total number of infected DC ($CD11c^+$) was related to the total number of parasites in the specific organ, a higher relative infection frequency of DC was observed in mice infected with type II parasites (Fig. 5C). We conclude that DC ($CD11c^+$) are preferentially parasitized early during infection and that mice inoculated with type II parasites exhibit a relatively higher infection frequency of DC than that exhibited by mice inoculated with type I.

Coinfections of mice with type I and type II strains show genotype-related differences in parasite distribution and dissemination. To further investigate differences in dissemination between type I and type II parasites, an experimental coinfection model was established. Mice were coinoculated with tachyzoites expressing GFP (type I, RH-LDM) and RFP (type II, PRU) (Fig. 6A) or with equivalent numbers of parasite CFU of infected DC (Fig. 6B). Extracellular (*e*) and intracellular (*i*) populations of type I (GFP^+) and type II (RFP^+) parasites were further studied by flow cytometry 16 h postinoculation (39). Coinoculation with free tachyzoites resulted in a strong domination of type I parasites (GFP^+) when the total parasite load in the spleen ($e + i$) was assessed (Fig. 6A), in line with previous results (Fig. 4A and B). Importantly, while type II (RFP^+) parasites were evenly distributed between intracellular and extracellular compartments, the type I (GFP^+) population was mainly extracellular (Fig. 6A, *e* versus *i*; Fig. 6C and D). This observation was also confirmed in mice infected with single strains (data not shown). In line with previous data (Fig. 4), coadoptive transfer of infected DC resulted in a significant increase in the total number of parasites reaching the spleen for type II (RFP^+) but only a slight increase in that

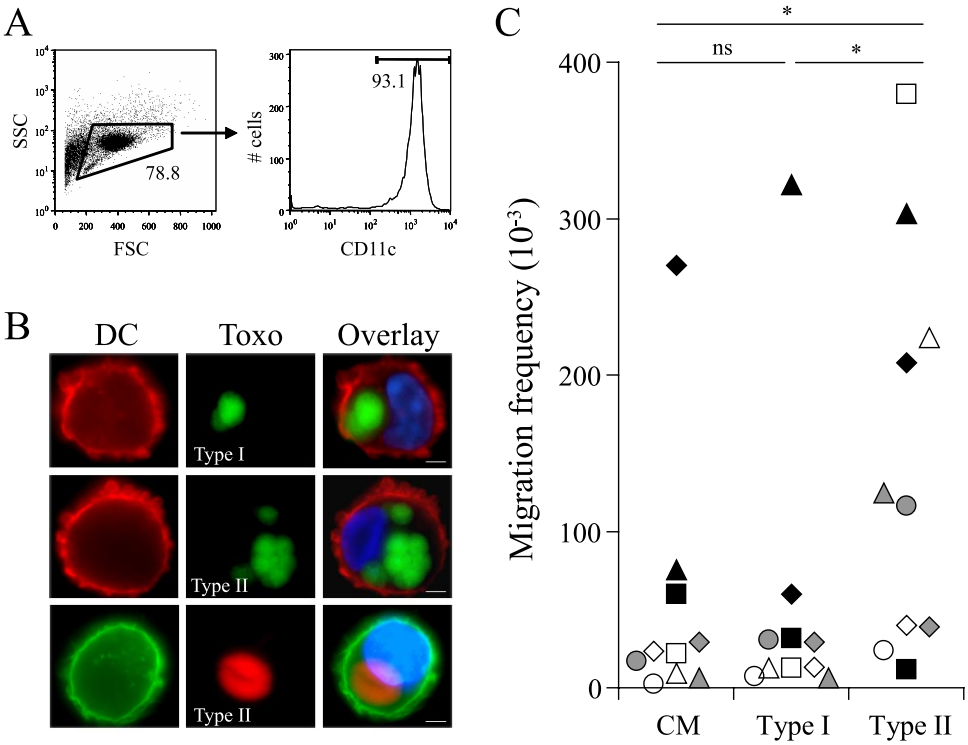

FIG. 3. Transmigration of primary human blood DC after *T. gondii* infection in vitro. Primary human myeloid DC were isolated from healthy blood donors as indicated in Materials and Methods. (A) A contour plot shows purified $CD1c^+$ cells, where the gate includes the dominating viable cell population. A histogram shows the proportion of $CD11c^+$ cells. (B) Immunofluorescence staining of human blood DC (phalloidin-Alexa Fluor 594 stain for top and middle panels; phalloidin-Alexa Fluor 488 stain for bottom panels) infected with *T. gondii* (type I GFP, RH-LDM; type II GFP, ME49-PTG; type II RFP, PRU). Overlay with DAPI (blue). Scale bar, 3 μm. (C) Transmigration frequency of primary human myeloid blood DC. DC were infected (MOI of 3) with tachyzoites (type I, RH-LDM; type II, ME49-PTG) for 2 h and incubated for 6 h in Transwell inserts. Transmigrated cells were quantified using a hematocytometer. Symbols represent DC from individual donors. An asterisk indicates significant difference ($P < 0.05$; paired *t* test).

number for type I (GFP^+) (Fig. 6, compare panels A and B, *e* + *i*). Importantly, this increased parasite load was strongly dominated by the intracellular fraction of the type II (RFP^+) parasite population (Fig. 6B, *e* versus *i*). Corroborating this observation, when coinoculation with free tachyzoites was compared to coinoculations with tachyzoite-infected DC, a significant increase in the intracellular fraction of parasites was observed for type II, but not for type I, populations (Fig. 6C and D). Next, DC were prelabeled to assess the migration of infected and uninfected DC to the spleen (Fig. 7A and B). A relative enrichment of infected DC over uninfected DC was observed in the spleen. Notably, this enrichment was more prominent for the DC population infected with type II parasites than for type I-infected DC (Fig. 7C). Altogether, this indicates that dissemination of type II parasites occurs preferentially in association with leukocytes, while for type I parasites, extracellular or nonhost cell-bound parasites likely predominate.

DISCUSSION

Rapid dissemination of parasites during primary toxoplasmosis is a key event that enables the establishment of infection in distant organs while effective immune control is gradually attained. Systemic dissemination is initialized within hours of infection, with accumulation in sites of immunological control like the spleen and lymph nodes in mice (3, 48, 51). In this study, dissemination of the three dominating *T. gondii* genotypes was assessed in vitro and in vivo. We provide evidence of genotype-linked preferences by means of parasite dissemination.

We recently reported that *T. gondii* subverts DC migration and induces a hypermotility phenotype that may promote parasite dissemination in vivo (30). The present studies establish that all strains tested induce transmigration of DC in vitro, suggesting a pivotal role for host cell-mediated parasite dissemination. The finding that the level of migratory induction in various types of DC was consistently related to the parasite genotype over time indicates an underlying genetic control of this trait. Moreover, type II (and III) strains consistently generated a superior induction of DC migration than type I strains. In contrast, extracellular tachyzoites of the type I genotype exhibit a potent transmigratory ability that likely facilitates passage across physiological barriers, a trait significantly less prominent in type II and III strains (3). Also, while high intracellular growth rates of type I parasites lead to heavy parasite tissue burdens and Th1-type cytokine overproduction in mice (17, 21, 36), type II parasites stimulate extensive interleukin-12 production (29, 42, 44), leading to immunological control of the infection. Thus, while type I parasites seem to rely on virulence traits for efficient parasite dissemination and

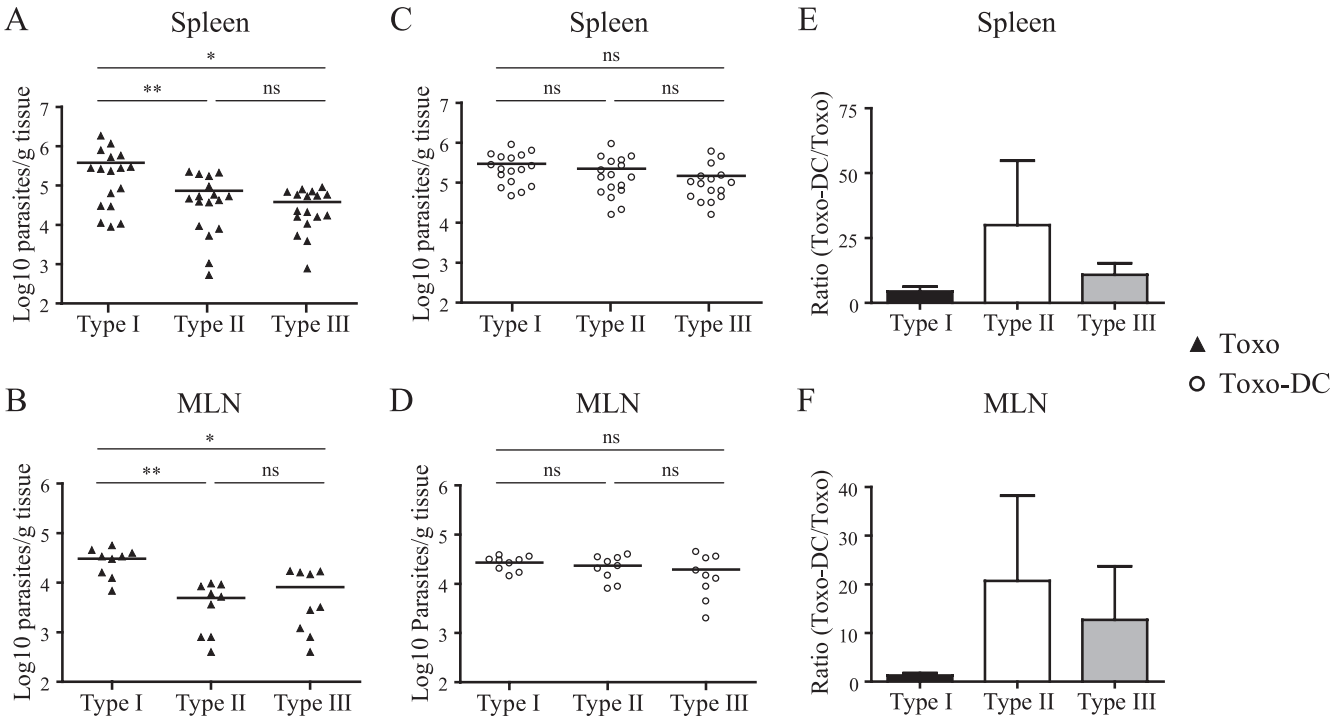

FIG. 4. Adoptive transfers of *Toxoplasma*-infected DC result in increased parasite loads. (A to D) C57BL/6 mice were inoculated i.p. with 10^6 CFU of freshly egressed tachyzoites (filled triangles) (A and B) or with 10^6 CFU of tachyzoite-infected DC (open circles) (C and D). Normalizations of inocula were performed as described in Materials and Methods. Parasite loads were quantified by a plaquing assay 16 h postinoculation. Representative strains were used for indicated genotypes (type I, RH-LDM; type II, ME49-PTG; type III, CTG). Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$; one-way ANOVA). ns, not significant. (E and F) The relative differences in parasite loads in mice infected with type I, II, or III strains were calculated for spleens and MLN. The relation of the magnitude of parasite load for each strain was determined as the ratio of the mean parasite loads after inoculation of tachyzoite-infected DC to the mean parasite loads after inoculation of free tachyzoites. Mean parasite tissue loads (black lines) from individual mice from three to four separate experiments are shown.

establishment of infection (3, 49), type II (and III) parasites may have refined their ability to utilize host cell migration to ensure efficient dissemination with a low parasitic load, resulting in minimized harm to the host.

Following oral infection, *T. gondii* penetrates the intestinal epithelium to infect cells in the subepithelial tissue (13, 14). Thus, intestinal DC are likely to be among the first cell populations parasitized. Accordingly, DC from the intestinal lamina propria were preferentially infected over other cell types and rapidly retrieved in circulation after oral infection (7). In our investigations of parasite dissemination at the initial phase of infection, the relatively low numbers of parasites crossing the intestinal mucosa after oral infection precluded quantifiable comparative analyses between strains. Nevertheless, we found high frequencies of infected $CD11c^+$ cells in spleens and MLN early after i.p. inoculation. In addition, induction of DC migration in vitro appears to set in rapidly (<3 h) after *T. gondii* invasion. Thus, the ability of type II parasites to rapidly induce a strong migratory phenotype in intestinal DC, and the preferential invasion of this cell type during the initial phase of infection, may facilitate early parasite dissemination and escape from the infection site.

Our findings demonstrate that primary blood DC from human donors can respond with a migratory phenotype upon *T. gondii* infection in vitro. Variations in the intensity of the response were observed between donors, and type II parasites generated a superior migratory response for DC. This is intriguing given the predominance of type II infections over other genotypes in clinical investigations (1, 16, 22–24) and motivates an elucidation of the genetic basis of *T. gondii*-induced cell migration. For immunocompromised individuals, e.g., for those with human immunodeficiency virus /AIDS, systemic dissemination of parasites strongly influences the outcome of severe primary infection or fulminant reactivated disease. While increased understanding has been attained in the murine infection model, the determinants of human infection remain elusive. It is also not clear why type II genotypes predominate in human toxoplasmosis (1, 16, 22–24). While strain-related differences in oral infectivity (47) and in immune responses (44) likely constitute important determinants, we argue that the strains that efficiently manage dissemination and establishment of dormant chronic infection in distant organs may also be most frequently associated with disease upon reactivation, e.g., type II genotypes. Also, the three archetypal *T. gondii* genotypes were tested in this study. Assessment of atypical strains exceeds this investigation but is motivated by their association with human disease (5, 11, 19).

While this study focuses on the role of DC, other cell types have been attributed a role in the dissemination of *T. gondii*, e.g., $CD11b^+$ monocytic cells (7), macrophages (8), T cells (39), and NK cells (38). It is therefore likely that multiple cell types function as Trojan horses during parasite dissemination.

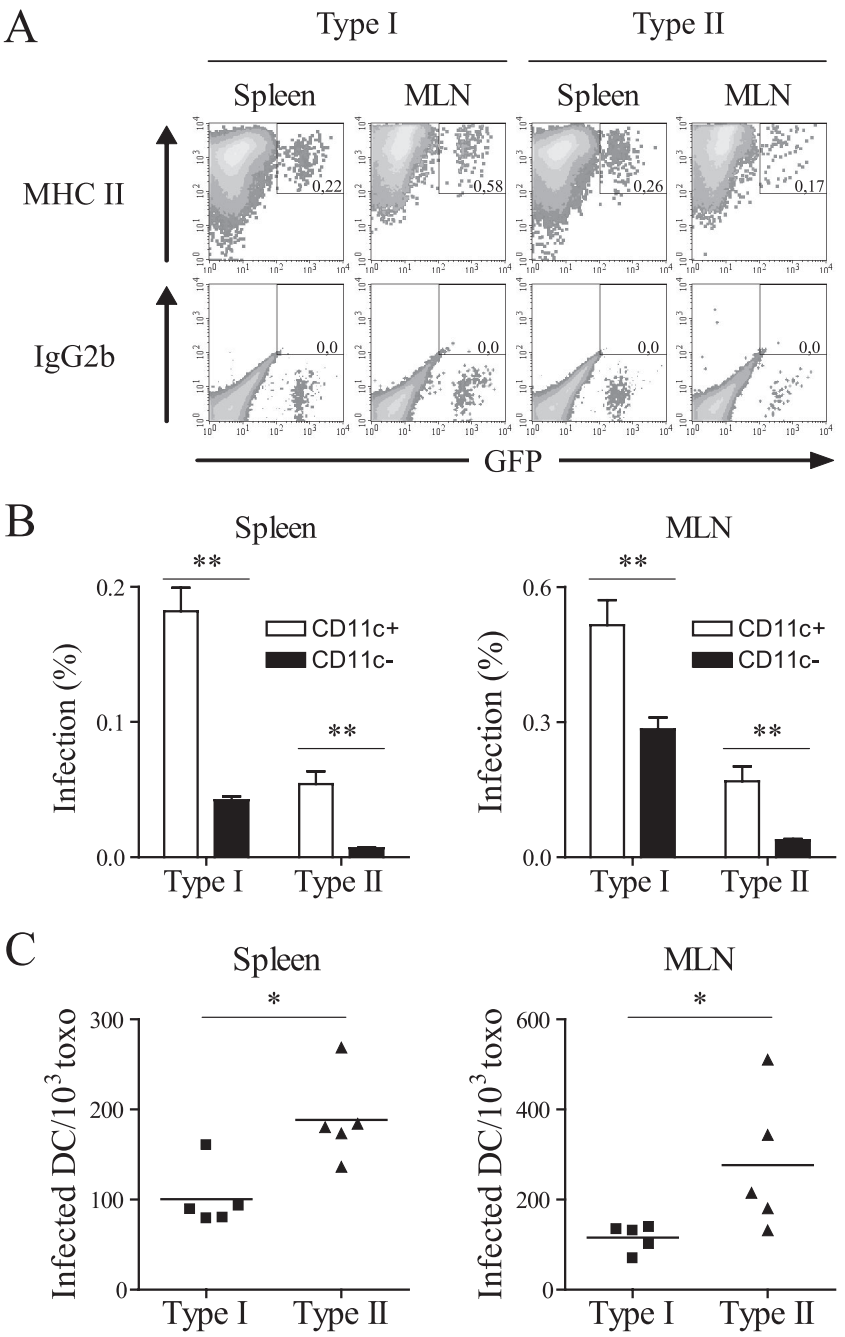

FIG. 5. DC are parasitized early during infection. C57BL/6 mice were inoculated i.p. with 2×10^6 CFU of freshly egressed GFP-transfected type I (RH-LDM) and type II (ME49-PTG) tachyzoites. After 32 h, $CD11c^+$ and $CD11c^-$ populations were isolated, and infection frequencies were evaluated by flow cytometry and plaquing assay as indicated in Materials and Methods. (A) Density plots show $CD11c^+$ cells from infected mice stained for MHC-II or an isotype control. Gates are set for infected (GFP^+) cells. (B) Bar diagrams (spleen and MLN) show infection frequencies (means ± standard errors of the means) of infected $CD11c^+$ ($CD3^-$, $CD19^-$, $Gr1^-$, $NK1.1^-$) and $CD11c^-$ cells for inoculations with type I and type II parasites, respectively. Asterisks indicate significant differences (**, $P < 0.01$; paired *t* test). (C) Diagrams (spleen and MLN) show the relative numbers of infected DC ($CD11c^+$, $CD3^-$, $CD19^-$, $Gr1^-$, $NK1.1^-$) per 10^3 parasites (toxo) for inoculations with type I and type II parasites, respectively. Black lines indicate the mean values for each group of five mice (triangles and squares). Asterisks indicate significant differences (*, $P < 0.05$; Student's *t* test). Results of a representative experiment with five mice/group are shown.

Yet, the relative significance and distinct roles of different leukocytic populations remain to be clarified; e.g., it has been suggested that macrophages may delay the dissemination process (8). Also, infiltrating $Gr1^+$ inflammatory monocytes participate in intestinal defense (15), and this cell type has the potential to differentiate into DC ($CD11c^+$) in vivo (18). Because monocytes/DC display phenotypic plasticity and CCR2-dependent mobilization (4, 15), future investigations of human blood-derived leukocytes will address whether their functional repertoire is shaped by parasite genotype-specific stimuli.

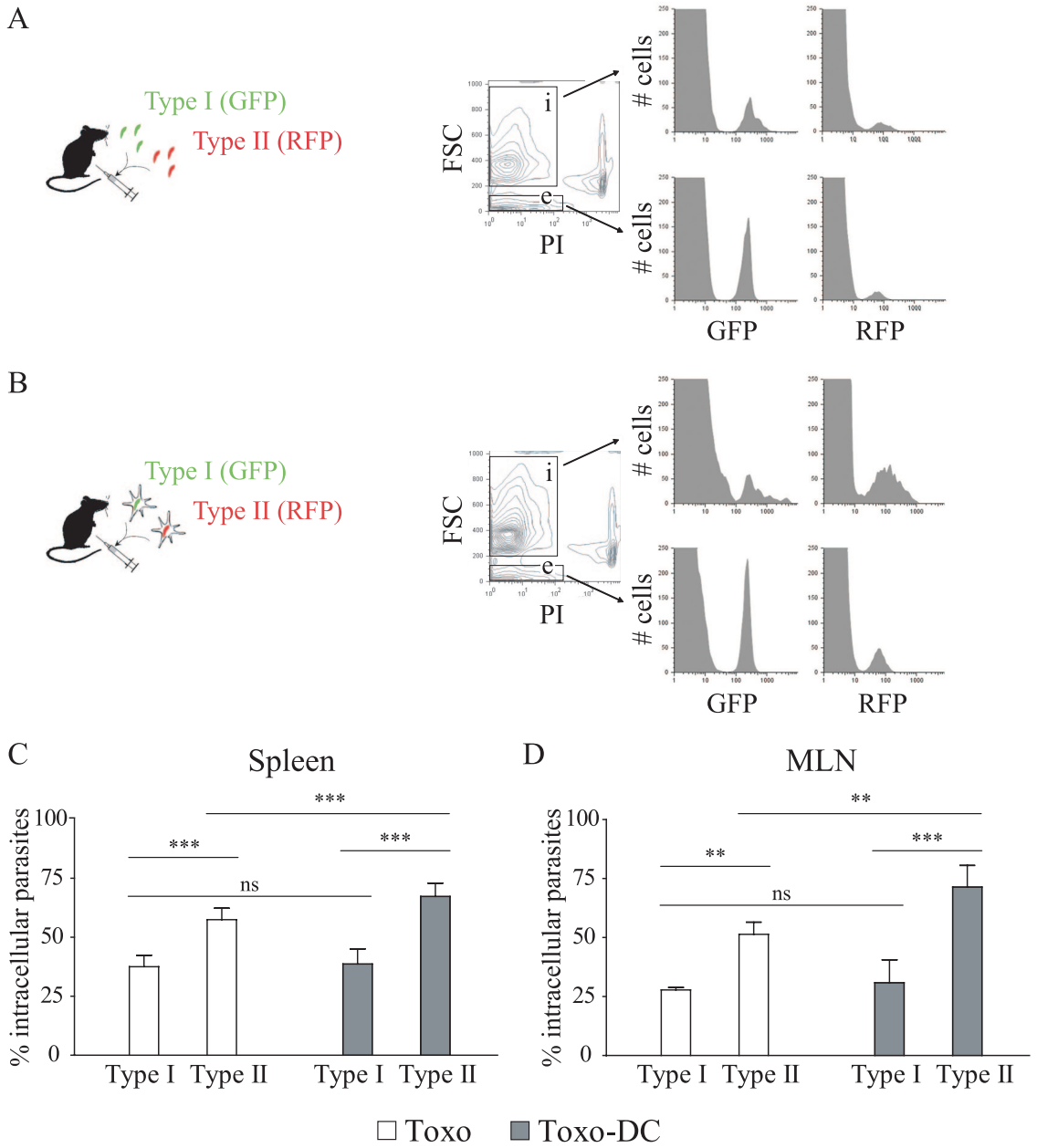

FIG. 6. Association with leukocytes characterizes the dissemination of type II parasites. (A) C57BL/6 mice were coinoculated i.p. with 2.5×10^6 CFU GFP-expressing type I (RH-LDM) tachyzoites and 2.5×10^6 CFU RFP-expressing type II (PRU) tachyzoites. Normalizations of inocula were performed as described in Materials and Methods. After 16 h, spleens were extracted, and cells were analyzed by flow cytometry. The gates in the plots include extracellular (*e*; FSC^{low}, PI^{neg}) and intracellular (*i*; FSC^{high}, PI^{neg}) parasites, respectively. Histograms show a disproportionate distribution of GFP^+ cells (type I), with a dominance of the extracellular fraction. In contrast, RFP^+ cells (type II) display an even distribution. FSC, forward scatter. (B) Bone marrow-derived DC were separately infected with type I (RH-LDM) and type II (PRU) tachyzoites in vitro. C57BL/6 mice were coinoculated i.p. with infected DC suspensions containing 2.5×10^6 CFU GFP-expressing type I (RH-LDM) and 2.5×10^6 CFU RFP-expressing type II (PRU) tachyzoites. Normalizations of inocula were performed as described in Materials and Methods. After 16 h, spleens were extracted, and cells were analyzed by flow cytometry. Histograms show a disproportionate distribution of GFP^+ cells (type I) similar to that in panel A. In contrast, RFP^+ cells (type II) show greater numbers and a strong dominance of the intracellular parasite fraction compared to the data displayed in panel A. (C and D) Bar diagrams show the mean (± standard deviation) percentage of intracellular parasites in the spleen and MLN, respectively, for the experimental setups described for panel A (Toxo) and panel B (Toxo-DC). Asterisks indicate significant differences (**, $P < 0.01$; ***, $P < 0.001$; paired *t* test and Student's *t* test for Toxo versus Toxo-DC). ns, not significant. Data from a representative experiment with eight mice/group are shown.

We found a predominance of infected $CD11c^+$ cells in the spleen and MLN early after i.p. inoculation, in line with observations in the lamina propria and MLN in an oral infection model (7). In addition, the circulating population of type II parasites was mainly confined intracellularly, while the extracellular parasite fraction predominated for type I parasites. In line with this, marginal effects on parasite dissemination were seen after adoptive transfer of DC infected with type I para-

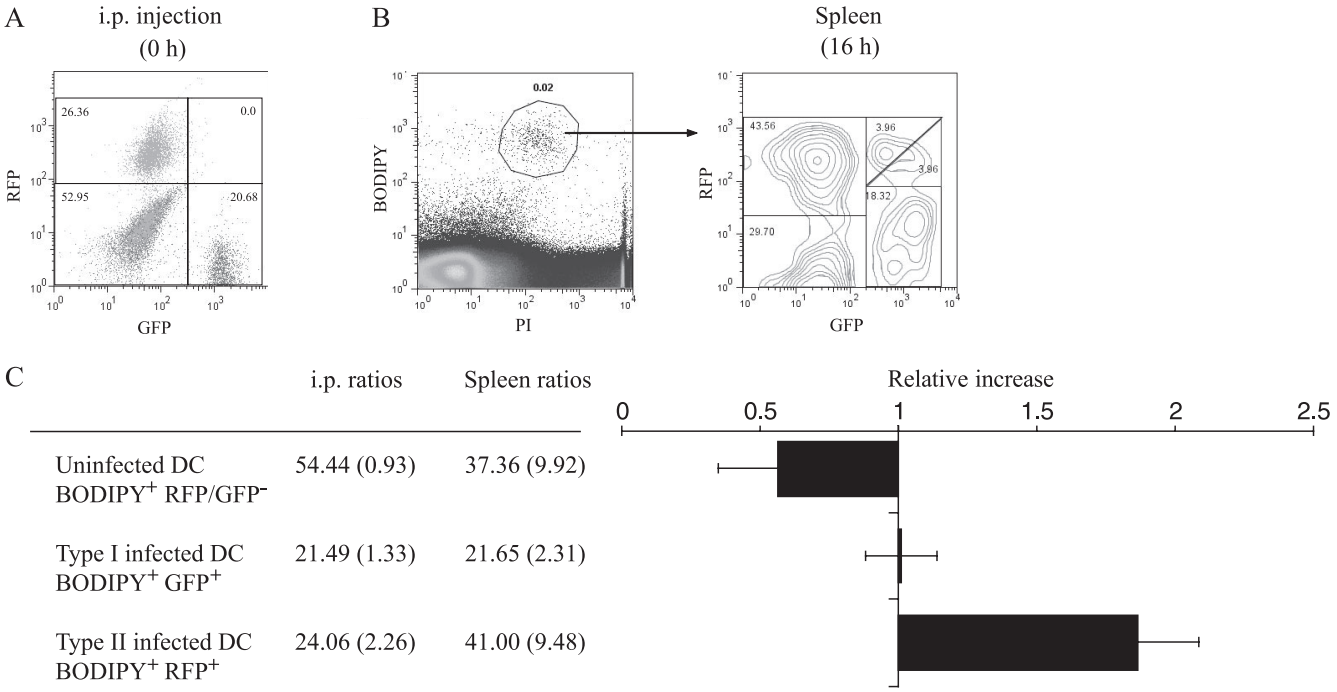

FIG. 7. Enrichment of migratory *Toxoplasma*-infected DC in the spleen after i.p. inoculation. DC were labeled with cell tracker (BODIPY B22802) and separately infected with type I (GFP^+, RH-LDM) or type II (RFP^+, PRU) parasites in vitro before i.p. coinoculation in C57BL/6 mice. After 16 h, spleens were extracted and disseminated BODIPY-positive ($BODIPY^+$) cells were assessed by flow cytometry after exclusion of nonviable cells (PI^+). (A) Plot shows the distribution of GFP^+ (type I), RFP^+ (type II), and GFP^-/RFP^- (uninfected) $BODIPY^+$ cells inoculated. (B) Plots show the distribution of GFP^+ (type I), RFP^+ (type II), GFP^+/RFP^+ (double infected), and GFP^-/RFP^- (uninfected) $BODIPY^+$ cells in the spleen. (C) Mean distribution ratios (±standard deviations) of the populations displayed in panels A and B at the time point of i.p. inoculation (i.p. ratios) and spleen extraction (spleen ratios). Bar diagram shows the mean (±standard deviation) relative increase coefficient for each population in the spleen related to the population in the peritoneal cavity. Data from two independent experiments are shown.

sites. In contrast, infected DC dramatically potentiated dissemination of type II parasites, and a high portion of type II-infected DC was found in peripheral organs in coinfections. Thus, type II (and III) parasites appear highly dependent on the shuttling function of DC for dissemination. Together, the cell type targeted for initial invasion, in combination with parasite genotype, may determine the kinetics of dissemination (7, 8, 30). Because innate immune responses to distinct parasite genotypes may influence the initial kinetics (<16 h) of dissemination upon coinfection, the differences in distribution of extracellular and intracellular parasites between type I and II infections were confirmed in mice infected with single strains.

Several invasive pathogens, e.g., bacteria, parasites, and virus (20, 34, 43), have been suggested to exploit DC migration to avoid clearance or to establish infection. The impact of this trait on the pathogenesis of infectious disease needs further clarification. Here, we present data indicating that *T. gondii* exploits, in a strain-specific manner, the host's cell trafficking machinery for parasite dissemination. Identification of the molecular components governing dissemination of infected DC may provide new insight into how pathogens manipulate host cells to the benefit of their propagation and persistence.

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